

Vitamin D Treatment during Pregnancy and Maternal and Neonatal Cord Blood Metal Concentrations at Delivery: Results of a Randomized Controlled Trial in Bangladesh

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BACKGROUND: Vitamin D improves absorption of calcium; however, in animal studies vitamin D also increases the absorption of toxic metals, such as lead and cadmium.

OBJECTIVES: We examined maternal and neonatal cord blood levels of lead, cadmium, manganese, and mercury after supplementation with vitamin D during pregnancy.

METHODS: The Maternal Vitamin D for Infant Growth trial was a randomized, placebo-controlled, multi-arm study of maternal vitamin D supplementation during pregnancy in Dhaka, Bangladesh (NCT01924013). Women were randomized during their second trimester to blinded weekly doses of placebo or 4,200, 16,800, or 28,000 IU of vitamin D3 throughout pregnancy. Each group had 118–239 maternal blood specimens and 100–201 cord blood samples analyzed. Metals were measured using inductively coupled plasma mass spectrometry. Unadjusted estimates from linear regression models were expressed as percentage differences. Cord blood cadmium was analyzed as detectable or undetectable with log-binomial regression.

RESULTS: Maternal cadmium, mercury, and manganese levels were nearly identical across groups. Maternal lead levels were 6.3%, 7.4%, and 6.0% higher in the treatment groups (4,200, 16,800, and 28,000 IU, respectively) vs. placebo; however, 95% confidence intervals (CIs) showed that differences from 4.1% lower to 20% higher were compatible with the data. In treatment groups (4,200, 16,800, 28,000 IU) vs. placebo, neonatal cord blood lead levels were 8.5% (95% CI: –3.5, 22), 16% (95% CI: 3.3, 30), and 11% (95% CI: 0.4, 23) higher and had higher risk of detectable cadmium, relative risk (RR) = 2.2 (95% CI: 1.3, 3.7), RR = 1.4 (95% CI: 0.8, 2.5), RR = 1.7 (95% CI: 1.0, 2.9).

DISCUSSION: Vitamin D supplementation from the second trimester of pregnancy did not influence maternal cadmium, mercury, or manganese levels at delivery. Vitamin D was associated with nonsignificant increases in maternal lead and with significant increases in cord blood lead and cadmium. These associations were not dose dependent. Given that there are no safe levels of metals in infants, the observed increases in cord blood lead and cadmium require further exploration. <https://doi.org/10.1289/EHP7265>

Introduction

Vitamin D is important for building healthy bones partly due to its role in stimulating calcium absorption. Adequate vitamin D is associated with improved absorption of not only calcium but also other essential elements, such as magnesium, iron, phosphate, zinc, and copper (Moon 1994). However, in addition to essential metals, in animal studies vitamin D treatment also increases the absorption of toxic metals, such as lead and cadmium (Goyer 1997; Moon 1994). In children, blood lead rises in the summer, and one proposed mechanism is the seasonal increase in vitamin D synthesis in the skin (Ngueta et al. 2015).

Calcium can interfere with toxic metal absorption [reviewed by Goyer (1997) and (Moon 1994)]. In fact, the Centers for Disease Control and Prevention (CDC) recommends calcium supplementation to prevent lead exposure in children (CDC 2002). Although

lead is the most well documented, calcium has also been reported to modulate the absorption of other toxic metals, such as cadmium (Schwalfenberg and Genuis 2015). Although calcium reduces the absorption of toxic metals, it may not fully prevent it, because vitamin D may also stimulate the uptake of toxic metals through a mechanism unrelated to calcium, for example through a passive process across intercellular spaces (Moon 1994).

Both the Endocrine Society (Holick et al. 2011) and the Institute of Medicine [Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium 2011] recommend that adults maintain a 25-hydroxyvitamin D (25OHD), the circulating serum or plasma biomarker of vitamin D [Holick et al. 2011; Hollis 2004; Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium 2011] level of at least 50 nmol/L. These recommendations have been criticized as being inadequate for pregnant women, with some authors suggesting that higher levels, 100–150 nmol/L, may be required to reduce the risk of pregnancy complications (Wagner and Hollis 2018). At the same time, high levels of 25OHD have also been associated with detrimental health outcomes, such as cancer and cardiovascular disease (Durup et al. 2015; Schwalfenberg and Genuis 2015). One proposed mechanism for this U-shaped association is vitamin D's hypothesized ability to stimulate the absorption of toxic metals (Schwalfenberg and Genuis 2015). Only one randomized trial has investigated whether vitamin D supplementation increases circulating levels of toxic metals in humans. This study of human immunodeficiency virus (HIV)-positive children and adolescents found that vitamin D treatment was not associated with increased blood lead, and that, in fact, the concomitant increase in 25OHD that occurred with vitamin D treatment was associated with a decrease in blood lead (Groleau et al. 2013). Mercury absorption has not been associated with vitamin D treatment in animals, but a

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recent observational study reported a nonsignificant increase in mercury with higher dietary vitamin D intake (Arbuckle et al. 2016). In total, it is unclear whether vitamin D treatment increases, decreases, or has no effect on circulating levels of toxic metals.

To our knowledge, no randomized trials of pregnant women have investigated vitamin D supplementation and levels of circulating metals. This is a critical research gap, given that maternal exposure to metals could have lasting consequences for infant growth or neurodevelopment (ATSDR 1999, 2007, 2012a). Cadmium (ATSDR 2012a), lead (ATSDR 2007), and mercury (ATSDR 1999) are toxicants that can cross the placenta and can cause several poor health outcomes, including low birth weight or growth restriction (ATSDR 2007, 2012a; Bloom et al. 2015; Johnston et al. 2014), preterm delivery (ATSDR 2007), and developmental delays (ATSDR 1999, 2007, 2012a; Kim et al. 2013; Yu et al. 2011), although for some metals the human studies are conflicting (ATSDR 2012a; Bloom et al. 2015). Maternal cadmium levels have been associated with reduced gestational age-adjusted birth weight and increased growth restriction in Bangladesh (Kippler et al. 2012a, 2012b). Early life cadmium has been associated with reduced weight and height at 5 years of age in Bangladesh (Gardner et al. 2013).

The aim of this study was to examine the blood levels of toxic metals (lead, cadmium, manganese, and mercury) in response to 4,200, 16,800, or 28,000 IU/wk of vitamin D supplementation during pregnancy (compared with placebo) administered during a randomized clinical trial in Bangladesh (Roth et al. 2015). If vitamin D supplementation increases levels of lead, cadmium, or mercury, it would suggest that high levels of supplementation be avoided by women seeking pregnancy or who are already pregnant.

Methods

Study Population

This is a secondary analysis from the Maternal Vitamin D for Infant Growth (MDIG) trial, which was a randomized, double-blinded, placebo-controlled, dose-ranging multi-arm study of maternal vitamin D supplementation during pregnancy in Dhaka, Bangladesh (NCT01924013). The original aim of the trial was to investigate the effect of maternal prenatal and postpartum vitamin D supplementation on infant length at 1 year of age. Women were recruited from government-funded health care facilities during their second trimester (17–24 wk gestation) from March 2014 to September 2015. Gestational age of pregnancy was based on the recalled last menstrual period estimate unless it differed by more than 5 d from a first trimester ultrasound if available or more than 10 d from the second trimester ultrasound performed by study technicians. Women were eligible if they were at least 18 years of age and intending to stay in the trial area for at least 18 months. They were ineligible if they had a medical condition that might alter vitamin D metabolism or if they had other risk factors, such as low hemoglobin, proteinuria, high blood pressure, multiple gestations, or congenital anomaly. Each participant received a blinded, prelabeled supplement packet according to a sequential unique identifier. The company that produced the supplement created the blinded packets using a random number sequence generated by, and known only to, the trial statistician according to a simple randomization scheme (i.e., there was no stratification or blocking). Further details about this trial have been published (Roth et al. 2015).

Participants in the trial were randomized to receive blinded weekly doses of 4,200, 16,800, or 28,000 IU of vitamin D3 (cholecalciferol) or placebo throughout pregnancy. Supplements were purchased from the Toronto Institute of Pharmaceutical Technology (TIPT). The TIPT does not market these supplements commercially.

Supplementation was administered weekly by trained study personnel either in the participant's home or in the clinic. In addition, women were offered 500 mg/d of calcium, 66 mg/d of elemental iron, and 350 µg/d of folic acid, the latter two being standard formulations available in Bangladesh. Adherence to routine calcium supplements was high, with >85% of participants reporting >85% adherence during a mid-trial audit. Mothers and infants were followed up routinely until the children were 2 years of age, with anthropometric measurements taken starting at birth.

The trial included 1,300 women, 260 in each arm (placebo, 4,200, and 16,000 IU/wk) except the 28,000 IU group, which had 520 women. The latter group was larger because half of the group received 28,000 IU prenatally and the other half received 28,000 IU both prenatally and postnatally.

Blood Collection

Trained phlebotomists collected maternal blood and cord venous blood specimens at delivery. Maternal samples were drawn into trace metal-free ethylenediaminetetraacetic acid (EDTA)-coated tubes and whole-blood aliquots were immediately drawn from each tube (before centrifugation). For the cord blood, as soon as possible after delivery (within 30 min), a site on the umbilical cord attached to the placenta was wiped with dry gauze to remove maternal blood. The umbilical vein was cannulated, and blood was collected into an EDTA lavender-top tube. Whole blood was stored at –70°C or colder in a freezer with a backup generator support and monitoring.

All women provided written informed consent that included the use of biospecimens in future research. The MDIG trial was approved by the research ethics board of the Hospital for Sick Children (Canada) and the ethical review committee of the International Center for Diarrheal Disease Research, Bangladesh. This nested substudy was approved by the research ethics board of the Hospital for Sick Children. The data analysis performed at the National Institute of Environmental Health Sciences was not considered to be human subjects research.

Measurement of Metals

The concentrations of maternal and umbilical whole-blood metals were measured in samples collected at the time of delivery. Cadmium, lead, mercury, and manganese were measured using inductively coupled plasma mass spectrometry at the CDC (Atlanta, GA) using validated, previously described laboratory methods (CDC 2012). Measurements of whole-blood metals are an important exposure biomarker because they reflect the amount of the metal circulating in the body from all environmental sources including air, water, and food and from internal stores during mobilization (CDC 2012).

Each analytical run was conducted using two (or three) levels of highly characterized quality control (QC) materials at the beginning and end of the run and was evaluated by an extended set of Westgard QC rules according to the division's QC requirements (Caudill et al. 2008). Each analytical method successfully participated in external Proficiency Testing (PT) programs according to Clinical Laboratory Improvement Amendments requirements. The analytical methods were validated using both historical PT materials as well as the National Institute of Standards and Technology (NIST) Standard Reference Materials or NIST traceable Reference Materials. Unused storage cryovials ($n = 25$) and caps were tested for the presence of manganese, mercury, cadmium, selenium, and lead using a standardized water-based leaching procedure (Ward et al. 2018). The vials did not contain significant levels of the analytes.

Of the 1,254 live births in the trial, some blood specimens were not available for metals analysis for the following reasons: Some women did not deliver at study hospitals, so blood could not be collected; plasma and serum were prioritized over whole blood, so if specimen volume was low, whole-blood aliquots may not have been generated; and some whole-blood samples could not be assayed due to micro-clotting. This left 619 maternal specimens and 516 cord blood specimens to be assayed (Figure 1). Each treatment group had 118 (45% of pregnant women originally enrolled in the MDIG), 141 (54%), 121 (47%), and 239 (46%) maternal blood specimens analyzed, respectively. Cord blood samples were analyzed for 100 (38%), 104 (40%), 111 (43%), and 201 (39%) pregnancies. A comparison of the women who had specimens measured for metals in this substudy and the MDIG participants who did not is presented in Table 1.

Measurement of 25OHD

Serum 25OHD concentrations were measured using liquid chromatography–tandem mass spectrometry at the Analytical Facility for Bioactive Molecules (AFBM) laboratory at the Hospital for Sick Children (Toronto, Canada). The AFBM participates in the Vitamin D External Quality Assessment Scheme Occasional Analyst certification program. Average inter-assay and intra-assay coefficients of variation were 10% and 5%, respectively. None of the samples were below the limit of quantification, which was 1.25 nmol/L.

Covariates

Given the randomized trial design, we did not hypothesize any adjustment factors *a priori*. However, covariates were included as stratification variables in sensitivity analyses (described in the section “Sensitivity Analysis”) and for description of the study

population. Covariates included were gestational age at enrollment, treatment adherence, smoking status, anemia, body mass index (BMI), fish consumption, water source, asset index, and month of enrollment (further described below).

Gestational age at enrollment was estimated using a combination of both last menstrual period and ultrasound. Vitamin D was dispensed at weekly study visits and treatment adherence was calculated as the percentage of scheduled prenatal vitamin D doses that were consumed. Smoking status was described using urinary cotinine level. Women with a urinary cotinine level of 50 ng/mL or less were considered nonsmokers and women above this cutoff were considered smokers (Kim 2016). Anemia was defined as a hemoglobin level at enrollment (~20 wk of gestation) of <104 g/L [Institute of Medicine (US) Committee on the Prevention Detection, and Management of Iron Deficiency Anemia Among U.S. Children and Women of Childbearing Age 1993]. Because women were enrolled during pregnancy, we did not have a measure of prepregnancy weight. Instead, BMI derived from height and weight measured at 12 months postpartum was used. Consumption of small, medium, and large fish over the past month was queried with a targeted dietary recall. Respondents described their frequency of consumption by choosing one of seven categories ranging from “never” to “more than 1 time per day: ____ times/day.” Water source was categorized as “piped into house/plot” and “deep tube well or bore hole.” Less than 0.5% of women reported their water source as “public tap” or “unknown,” and they were excluded from analyses that included water source. Water source may be associated with metals levels if it is also related to the amount of surface vs. groundwater present because those types of water differ in sources of pollution and metals levels (Hasan et al. 2019). An asset index was constructed from a baseline survey on household characteristics (Roth et al. 2018). In brief, ownership (yes/no) of the following 19 items was self-reported: private toilet,

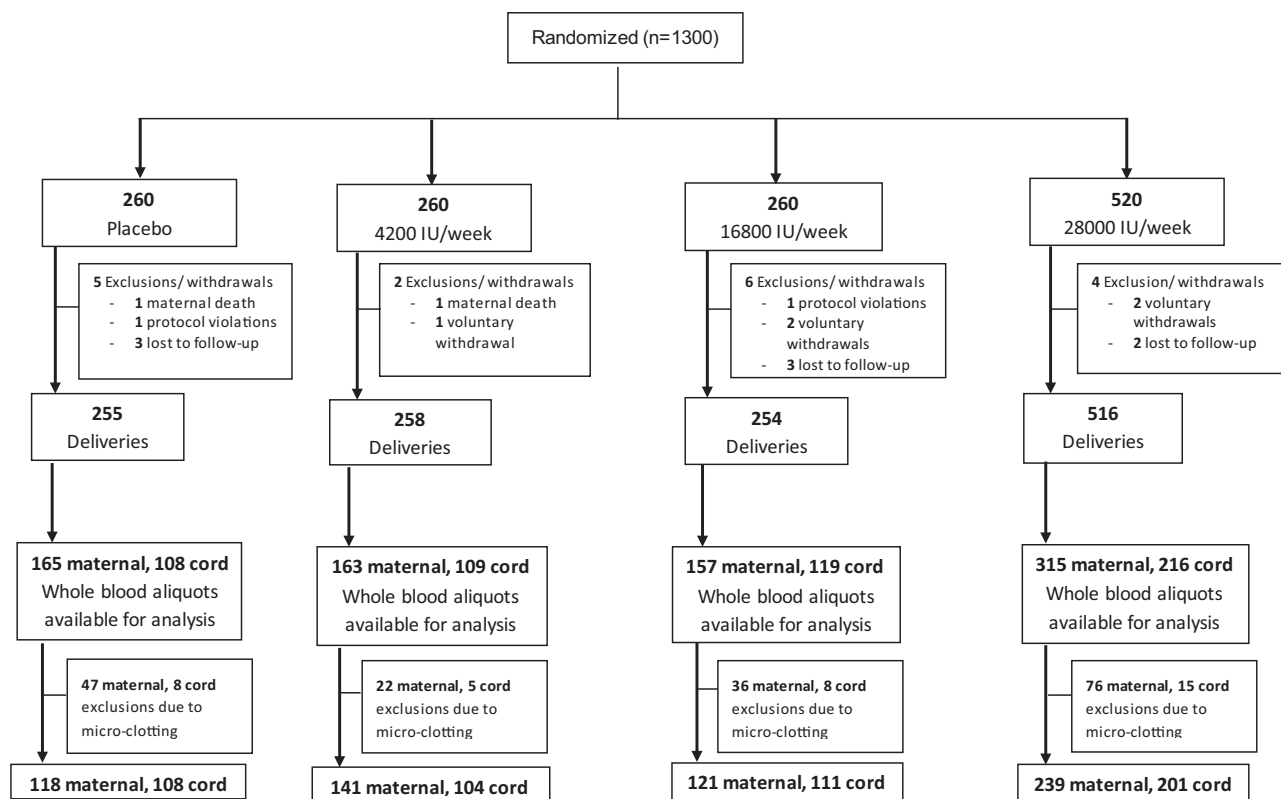


Figure 1. CONSORT diagram showing the maternal and cord samples from the MDIG trial that were analyzed for metal levels at the CDC. Note: CDC, Centers for Disease Control and Prevention; CONSORT, Consolidated Standards of Reporting Trials; MDIG, Maternal Vitamin D for Infant Growth.

Table 1. Maternal characteristics [*n* (%)] at enrollment among women in the entire MDIG trial compared with women in the metals substudy.

Characteristics	Participants without metals data	Participants with delivery and cord metals, or both	<i>p</i> -Value ^a
Enrolled participants (<i>n</i>)	679	619	
Gestational age at enrollment (wk)			0.91
<19	198 (29.2)	170 (27.5)	
19–20	111 (16.3)	107 (17.3)	
21–22	201 (29.6)	188 (30.4)	
>22	169 (24.9)	154 (24.9)	
Age (y)			0.21
18–20	258 (38.0)	204 (33.0)	
21–25	251 (37.0)	252 (40.7)	
26–30	141 (20.8)	129 (20.8)	
>30	29 (4.3)	34 (5.5)	
Education			0.62
No schooling	33 (4.9)	25 (4.0)	
Primary incomplete	150 (22.1)	127 (20.5)	
Primary complete	89 (13.1)	90 (14.5)	
Secondary incomplete	266 (39.2)	232 (37.5)	
Secondary complete	141 (20.8)	145 (23.4)	
Asset index (quintile) ^b			0.94
1	139 (20.9)	122 (19.8)	
2	128 (19.3)	123 (20.0)	
3	128 (19.3)	128 (20.8)	
4	134 (20.2)	123 (20.0)	
5	135 (20.3)	120 (19.5)	
Employment ^c			0.65
Homemaker	622 (93.4)	580 (94.0)	
Other	44 (6.6)	37 (6.0)	
Gravidity ^d			0.22
1	266 (39.2)	218 (35.2)	
2	214 (31.5)	218 (35.2)	
3	146 (21.5)	123 (19.9)	
≥4	53 (7.8)	60 (9.7)	
Baseline 25OHD (nmol/L)			0.84
<20	227 (33.6)	215 (34.9)	
20–<40	325 (48.1)	300 (48.7)	
40–<50	74 (11.0)	62 (10.1)	
≥50	49 (7.3)	39 (6.3)	
Treatment adherence (%)			0.001
≤95	141 (20.8)	43 (6.9)	
>95	538 (79.2)	576 (93.1)	
Smoking status (by cotinine) ^e			0.001
Missing	669 (98.5)	200 (32.3)	
Nonsmoker	10 (1.5)	399 (64.5)	
Smoker	0 (0.0)	20 (3.2)	
BMI (kg/m ²) ^f			0.001
Missing (<i>n</i>)	119 (17.5)	57 (9.2)	
<18.5	67 (9.9)	59 (9.5)	
18.5–25	305 (44.9)	276 (44.6)	
>25–30	140 (20.6)	180 (29.1)	
>30	48 (7.1)	47 (7.6)	
Anemia (Hb <104 g/L)			0.32
No	421 (62.0)	367 (59.3)	
Yes	258 (38.0)	252 (40.7)	
Water source ^c			0.02
Piped into house/plot	509 (76.4)	511 (82.8)	
Public tap	3 (0.5)	2 (0.3)	
Deep tube well or bore hole	150 (22.5)	103 (16.7)	
Unknown	4 (0.6)	1 (0.2)	
Month of enrollment			0.001
March–May	196 (28.9)	269 (43.5)	
June–August	175 (25.8)	237 (38.3)	
September–November	148 (21.8)	77 (12.4)	
December–February	160 (23.6)	36 (5.8)	
Protein consumption (tertile)			0.62
Lowest	193 (28.4)	191 (30.9)	
Middle	219 (32.3)	196 (31.7)	
Highest	267 (39.3)	232 (37.5)	

Table 1. (Continued.)

Characteristics	Participants without metals data	Participants with delivery and cord metals, or both	<i>p</i> -Value ^a
Small fish consumption			0.02
Never	184 (27.1)	207 (33.4)	
1–3 times a month	207 (30.5)	200 (32.3)	
Once a week	86 (12.7)	67 (10.8)	
≥2 times a week	202 (29.7)	145 (23.4)	
Medium fish consumption			0.02
Never	245 (36.1)	182 (29.4)	
1–3 times a month	144 (21.2)	170 (27.5)	
Once a week	68 (10.0)	61 (9.9)	
≥2 times a week	222 (32.7)	206 (33.3)	
Large fish consumption			0.004
Never	118 (17.4)	124 (20.0)	
1–3 times a month	185 (27.2)	203 (32.8)	
Once a week	90 (13.3)	91 (14.7)	
≥2 times a week	286 (42.1)	201 (32.5)	

Note: BMI, body mass index; Hb, hemoglobin; MDIG, Maternal Vitamin D for Infant Growth; *n*_{CD-study}, number in sample from the cadmium study; *n*_{MDIG}, number in sample from Maternal Vitamin D for Infant Growth; 25OHD, 25-hydroxyvitamin D.

^aParametric and nonparametric tests were used to assess any differences between groups (*t*-test, Mann-Whitney *U* test, chi-square test); metals sub-study participants were compared to MDIG participants who were not eligible for inclusion in the metals study (*n* = 679).

^b*n*_{MDIG} = 664, *n*_{Metals sub-study} = 616.

^c*n*_{MDIG} = 666, *n*_{Metals sub-study} = 617.

^dNumber of pregnancies, including the current pregnancy.

^eUrine cotinine was measured among women with blood samples available for metals analysis.

^f*n*_{MDIG} = 560, *n*_{Metals sub-study} = 562.

electricity, radio, TV, mobile phone, nonmobile phone, fridge, almirah/wardrobe, table, chair, electric fan, DVD/CD player, auto-bike, rickshaw/van, bicycle, motorcycle/motor scooter/tempo/CNG (motorized rickshaw), livestock/herds/farm animals/poultry, homestead, and land. The first principal component from a principal component analysis was used to assign each participant an asset score (lower scores reflect less wealth). This asset index was categorized into quintiles for the analysis.

Statistical Analysis

Metal levels were natural log-transformed for analysis. We calculated median, 25th, and 75th percentiles of metal concentration for each treatment group and in both maternal and cord blood samples. We used an unadjusted, intention-to-treat, linear regression to compare metal levels across treatment groups. Beta estimates from the regression model were exponentiated and one was subtracted from that number, then each result was multiplied by 100 and expressed as the percentage difference. Likelihood ratio *p*-values were calculated for a model with the three indicator variables for treatment group and without. Many of the neonatal cord blood cadmium levels were below the limit of detection (LOD; 0.1 µg/L; 76%), and, thus, cadmium was dichotomized into detectable and undetectable and analyzed with a log-binomial regression model. One maternal sample was below the LOD for lead and this was imputed as the LOD for analysis. The LODs for the other metals were as follows: manganese = 0.99 µg/L, lead = 0.1 µg/L, and mercury = 0.28 µg/L. None of the other samples were below their LOD.

Sensitivity Analyses

First, we investigated the potential interaction between baseline 25OHD and metals level at delivery by examining *a*) the Spearman correlation between baseline 25OHD and natural log-transformed metal levels at delivery; *b*) the Spearman correlation between natural log-transformed metals levels and the change

Table 2. Demographic and behavioral characteristics at enrollment, stratified by vitamin D treatment group.

Characteristics	Treatment group (IU/wk)										<i>p</i> -Value
	Overall		Placebo		4,200		16,800		28,000		
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)	
Enrolled participants (<i>n</i>) ^a	619		118		141		121		239		
Gestational age at enrollment (wk)											
<19	170	(27.5)	26	(22.0)	41	(29.1)	34	(28.1)	69	(28.9)	0.32
19–20	107	(17.3)	20	(16.9)	32	(22.7)	18	(14.9)	37	(15.5)	
21–22	188	(30.4)	34	(28.8)	37	(26.2)	37	(30.6)	80	(33.5)	
>22	154	(24.9)	38	(32.2)	31	(22.0)	32	(26.4)	53	(22.2)	
Age (y)											
18–20	204	(33.0)	45	(38.1)	43	(30.5)	40	(33.1)	76	(31.8)	0.44
21–25	252	(40.7)	40	(33.9)	61	(43.3)	53	(43.8)	98	(41.0)	
26–30	129	(20.8)	25	(21.2)	25	(17.7)	24	(19.8)	55	(23.0)	
>30	34	(5.5)	8	(6.8)	12	(8.5)	4	(3.3)	10	(4.2)	
Education											
No schooling	25	(4.0)	3	(2.5)	4	(2.8)	5	(4.1)	13	(5.4)	0.36
Primary incomplete	127	(20.5)	26	(22.0)	26	(18.4)	25	(20.7)	50	(20.9)	
Primary complete	90	(14.5)	18	(15.3)	21	(14.9)	12	(9.9)	39	(16.3)	
Secondary incomplete	232	(37.5)	42	(35.6)	48	(34.0)	57	(47.1)	85	(35.6)	
Secondary complete	145	(23.4)	29	(24.6)	42	(29.8)	22	(18.2)	52	(21.8)	
Asset index (quintile)											
1	122	(19.7)	27	(22.9)	27	(19.1)	15	(12.4)	53	(22.2)	0.65
2	123	(19.9)	20	(16.9)	31	(22.0)	28	(23.1)	44	(18.4)	
3	128	(20.7)	25	(21.2)	25	(17.7)	30	(24.8)	48	(20.1)	
4	123	(19.9)	25	(21.2)	25	(17.7)	26	(21.5)	47	(19.7)	
5	120	(19.4)	21	(17.8)	32	(22.7)	22	(18.2)	45	(18.8)	
Employment											
Homemaker	580	(93.7)	113	(95.8)	133	(94.3)	112	(92.6)	222	(92.9)	0.76
Other	37	(6.0)	5	(4.2)	8	(5.7)	9	(7.4)	15	(6.3)	
Gravidity ^b											
1	218	(35.2)	40	(33.9)	52	(36.9)	45	(37.2)	81	(33.9)	0.47
2	218	(35.2)	43	(36.4)	47	(33.3)	34	(28.1)	94	(39.3)	
3	123	(19.9)	20	(16.9)	32	(22.7)	27	(22.3)	44	(18.4)	
≥4	60	(9.7)	15	(12.7)	10	(7.1)	15	(12.4)	20	(8.4)	
Baseline 25OHD (nmol/L)											
<20	215	(34.7)	38	(32.2)	48	(34.0)	39	(32.2)	90	(37.7)	0.21
20–<40	300	(48.5)	61	(51.7)	71	(50.4)	58	(47.9)	110	(46.0)	
40–<50	62	(10.0)	15	(12.7)	15	(10.6)	10	(8.3)	22	(9.2)	
≥50	39	(6.3)	3	(2.5)	6	(4.3)	14	(11.6)	16	(6.7)	
Treatment adherence (%)											
<95	37	(6.0)	8	(6.8)	6	(4.3)	8	(6.6)	15	(6.3)	0.80
≥95	582	(94.0)	110	(93.2)	135	(95.7)	113	(93.4)	224	(93.7)	
Smoking status (by cotinine)											
Missing	200	(32.3)	38	(32.2)	52	(36.9)	43	(35.5)	67	(28.0)	0.57
Nonsmoker	399	(64.5)	77	(65.3)	85	(60.3)	76	(62.8)	161	(67.4)	
Smoker	20	(3.2)	3	(2.5)	4	(2.8)	2	(1.7)	11	(4.6)	
BMI (kg/m ²)											
Missing	57	(9.2)	13	(11.0)	14	(9.9)	4	(3.3)	26	(10.9)	0.41
<18.5	59	(9.5)	13	(11.0)	15	(10.6)	10	(8.3)	21	(8.8)	
18.5–25	276	(44.6)	57	(48.3)	66	(46.8)	50	(41.3)	103	(43.1)	
>25–30	180	(29.1)	28	(23.7)	33	(23.4)	48	(39.7)	71	(29.7)	
>30	47	(7.6)	7	(5.9)	13	(9.2)	9	(7.4)	18	(7.5)	
Anemia (Hb <104 g/L)											
No	367	(59.3)	66	(55.9)	85	(60.3)	69	(57.0)	147	(61.5)	0.72
Yes	252	(40.7)	52	(44.1)	56	(39.7)	52	(43.0)	92	(38.5)	
Water source											
Piped into house/plot	511	(82.6)	96	(81.4)	105	(74.5)	105	(86.8)	205	(85.8)	0.05
Public tap	2	(0.3)	0	0	0	0	0	0	2	(0.8)	
Deep tube well or bore hole	103	(16.6)	22	(18.6)	36	(25.5)	16	(13.2)	29	(12.1)	
Unknown	1	(0.2)	0	0	0	0	0	0	1	(0.4)	
Month of enrollment											
March–May	269	(43.5)	52	(44.1)	57	(40.4)	54	(44.6)	106	(44.4)	1.0
June–August	237	(38.3)	45	(38.1)	58	(41.1)	45	(37.2)	89	(37.2)	
September–November	77	(12.4)	14	(11.9)	16	(11.3)	16	(13.2)	31	(13.0)	
December–February	36	(5.8)	7	(5.9)	10	(7.1)	6	(5.0)	13	(5.4)	
Small fish consumption											
Never	207	(33.4)	37	(31.4)	51	(36.2)	38	(31.4)	81	(33.9)	0.68
1–3 times a month	200	(32.3)	36	(30.5)	48	(34.0)	40	(33.1)	76	(31.8)	
Once a week	67	(10.8)	13	(11.0)	13	(9.2)	19	(15.7)	22	(9.2)	
≥2 times a week	145	(23.4)	32	(27.1)	29	(20.6)	24	(19.8)	60	(25.1)	

Table 2. (Continued.)

Characteristics	Treatment group (IU/wk)										p-Value
	Overall		Placebo		4,200		16,800		28,000		
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	
Medium fish consumption											
Never	182	(29.4)	29	(24.6)	47	(33.3)	37	(30.6)	69	(28.9)	0.23
1–3 times a month	170	(27.5)	44	(37.3)	33	(23.4)	32	(26.4)	61	(25.5)	
Once a week	61	(9.9)	9	(7.6)	13	(9.2)	17	(14.0)	22	(9.2)	
≥2 times a week	206	(33.3)	36	(30.5)	48	(34.0)	35	(28.9)	87	(36.4)	
Large fish consumption											
Never	124	(20.0)	21	(17.8)	27	(19.1)	21	(17.4)	55	(23.0)	0.32
1–3 times a month	203	(32.8)	40	(33.9)	54	(38.3)	34	(28.1)	75	(31.4)	
Once a week	91	(14.7)	12	(10.2)	18	(12.8)	22	(18.2)	39	(16.3)	
≥2 times a week	201	(32.5)	45	(38.1)	42	(29.8)	44	(36.4)	70	(29.3)	

Note: BMI, body mass index; Hb, hemoglobin; 25OHD, 25-hydroxyvitamin D.

^aNumbers in other rows may not add to totals due to missing values.

^bChi-square *p*-value, calculated without missing values where applicable.

^cNumber of pregnancies, including the current pregnancy.

in 25OHD from baseline to delivery; and *c*) the treatment group effects after excluding women with sufficient 25OHD (≥ 50 nmol/L) at baseline. Second, we examined whether results differed if analyses were limited to women with at least 95% adherence to treatment. Third, we further explored the associations between vitamin D treatment and metals after adjusting for water source, BMI, and month. These were included based on hypotheses that *a*) water source may provide different levels of metals exposure (as previously described in the section “Covariates”); *b*) vitamin D treatment effects may be modulated by BMI (Holick et al. 2011); and *c*) there are monthly patterns to metal exposure in Bangladesh (Hasan et al. 2019). We also examined the interaction between vitamin D treatment and BMI through a stratified analysis. Interaction *p*-values were calculated by entering an interaction term between treatment group and a dichotomous indicator variable for obese/overweight vs. normal/underweight in the model.

Results

Descriptive Characteristics

Compared with the full MDIG cohort, women and infants that had metals data were more likely to have high treatment adherence, which occurred by design, given that metals were measured in delivery blood, which required women to stay in the study until delivery (Table 1). Similarly, women and infants with metals data were also more likely to have BMI information, again, most likely because BMI was not measured until 12 months postpartum. Participants with metals data were more likely to have their water piped into the house and were more likely to have enrolled between March and May (and less likely to have enrolled between December and February). They were also more likely to never eat small-sized fish, to eat medium-sized fish, and to less frequently eat large-sized fish.

This analysis was conducted among a subset of women who had biospecimens available for metals measurement. Although the full cohort was randomized, it was possible that maternal characteristics were not equally distributed across groups in this subset. For the most part, characteristics were balanced across treatment groups. One variable did show some difference: Women who reported having a deep tube well as their water source were more likely to be in the placebo or lowest treatment groups (chi-square $p = 0.0063$) (Table 2). None of the other variables differed across treatment groups ($p > 0.1$).

Lead levels were high in this population, with 90% of maternal levels and 82% of cord blood levels being above the reference

value from the CDC (5 $\mu\text{g}/\text{dL}$) (USPSTF et al. 2019) (Table 3). In maternal blood, 20% of cadmium values were above the reference value (1.0 $\mu\text{g}/\text{L}$) (Schulz et al. 2007), whereas three of the neonatal cord blood values exceeded the reference level for children (0.5 $\mu\text{g}/\text{L}$). Six maternal mercury levels and 5% of neonatal cord blood levels were higher than the reference value [4.6 $\mu\text{g}/\text{L}$ (Brodin et al. 2007)]. Manganese does not have a reference level for comparison.

Maternal and neonatal cord blood metals were correlated: lead, $r = 0.84$ ($p < 0.0001$), manganese, $r = 0.25$ ($p < 0.0001$), mercury, $r = 0.083$ ($p < 0.0001$), cadmium, $r = 0.31$ ($p = 0.002$).

Vitamin D Treatment and Metals Levels

Median maternal and neonatal cord blood lead levels were higher in the vitamin D supplementation groups than in the placebo group (Table 3). In the intent-to-treat analysis, compared with placebo, maternal lead levels were 6.3% (−4.1, 18), 7.4% (−3.5, 20), and 6.0% (−3.4, 16) higher in the 4,200-, 16,800-, and 28,000-IU/wk treatment groups, respectively, with a likelihood ratio test (LRT) *p*-value of 0.54 comparing models with indicator variables for treatment groups and without (Table 4). For neonatal cord blood lead, compared with placebo, the vitamin D supplementation groups had levels that were 8.5% (−3.5, 22), 16% (3.3, 30), and 11% (0.4, 23) higher, respectively (LRT, 3 degrees of freedom, $p = 0.08$) (Table 4). The vitamin D supplementation groups also had a higher risk of having detectable cord blood cadmium levels compared with placebo. The percentage differences of maternal cadmium and cord blood mercury were positive for each treatment group vs. placebo, but not statistically significant and the magnitudes were small relative to the width of their confidence intervals, and there was no evidence of a dose-response effect. Treatment group differences for maternal mercury, maternal manganese, and cord blood manganese were small (close to null) and inconsistent in direction (Table 4).

Sensitivity Analyses

At baseline, the median 25OHD level was 25 nmol/L (interquartile range: 17–34). As expected, women with higher levels of 25OHD at baseline showed a smaller increase in 25OHD across the study (Table 5). For the most part, baseline 25OHD levels were not correlated with metal levels at delivery. However, baseline 25OHD was correlated with maternal mercury levels and with neonatal cord blood mercury levels (Table 5). Consistent with the primary results, the change in 25OHD from baseline to delivery was also correlated with increased cord blood lead (Table 5). After excluding 39 women with sufficient 25OHD

Table 3. Metal levels in maternal delivery and neonatal cord blood samples, stratified by treatment group.

Samples	Treatment group (IU/wk)				
	Overall	Placebo	4,200	16,800	28,000
Metal (IQR)					
Maternal samples (<i>n</i>)	619	118	141	121	239
Cord blood samples (<i>n</i>)	516	100	104	111	201
Metal [median (IQR)]					
Lead (μg/dL)					
Maternal ^a	9.0 (6.7–12)	8.6 (6.1–11)	9.0 (7.0–12)	9.6 (6.6–13)	9.2 (6.8–12)
Percentage above reference value ^b	90	86	92	90	91
Cord ^c	7.6 (5.7–10)	6.7 (5.0–9.1)	7.6 (5.8–9.6)	8.3 (5.8–11)	7.7 (6.0–10)
Percentage above reference value ^b	82	75	85	82	85
Cadmium (μg/dL)					
Maternal ^c	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.7 (0.5–0.9)	0.7 (0.5–0.9)
Percentage above reference value ^b	20	18	23	20	19
Cord ^d	24% (124)	15% (15)	33% (34)	21% (23)	26% (52)
Percentage above reference value ^b	0.6	2.0	0.0	0.9	0.0
Mercury (μg/dL) ^e					
Maternal	1.2 (0.9–1.5)	1.2 (0.9–1.6)	1.1 (0.9–1.5)	1.1 (1.0–1.6)	1.2 (0.9–1.5)
Percentage above reference value ^b	1.0	0.8	1.4	1.6	0.4
Cord	2.2 (1.7–3.0)	2.1 (1.6–2.9)	2.1 (1.8–2.9)	2.4 (1.7–3.1)	2.3 (1.8–3.0)
Percentage above reference value ^b	4.8	5.0	3.8	5.4	5.0
Manganese (μg/dL)					
Maternal	18 (14–21)	18 (14–21)	18 (14–21)	18 (15–22)	17 (14–22)
Cord	40 (32–50)	40 (32–53)	38 (31–48)	40 (32–50)	41 (34–50)

Note: IQR, interquartile range; LOD, limit of detection.

^aOne sample below the LOD was imputed as the LOD (0.1 μg/L).

^bReference values for each metal were lead: 5 μg/dL, cadmium: 1.0 μg/L for adults and 0.5 μg/L for children, mercury: 4.6 μg/L. Manganese does not have an accepted reference level.

^cThe LODs for the metals were cadmium = 0.1 μg/L, manganese = 0.99 μg/L, lead = 0.1 μg/L, and mercury = 0.28 μg/L. None of the cord blood lead, maternal cadmium, mercury, or manganese values were below the LOD.

^d76% of samples were undetectable, numbers shown are proportion detectable (*n*).

(≥50 nmol/L) at baseline, percentage differences in cord blood mercury from pregnancies in the treatment vs. placebo groups were slightly higher, with little to no change in the remaining point estimates or their precision; thus, our interpretation of the primary results did not change (Table S1). Similarly, when excluding women who received less than 95% of their scheduled treatment doses, the results were unchanged other than the association between treatment group and cord blood lead level, which was slightly stronger (Table S1).

Women who reported that their water source was a deep tube well had slightly higher cord blood lead levels than women who reported that their water was pumped into their house or onto their land [geometric mean (GM) 8.0 vs. 7.3 μg/L, respectively, analysis of variance (ANOVA) *p* = 0.09]. Maternal GM lead levels were higher in overweight and obese women compared with normal and underweight women (ANOVA *p* = 0.0003; Table 6).

Metal levels differed across month of enrollment. Maternal and neonatal lead and mercury were highest from June to November, and lowest from December to May (Table S2). Maternal cadmium was highest between December and February, whereas infant cord blood cadmium was more likely to be detectable from September to February. Multivariable adjustment for water source, month of enrollment, and BMI did not alter our interpretations of the primary findings (Table S1). The percentage differences were larger with multivariable adjustment for maternal lead and for cord blood lead with the exception of the lowest treatment group, which was 0.1% smaller (Table S1). For mercury, manganese, and maternal cadmium the multivariable-adjusted percentage differences were small (<5.1% for all treatment groups) and with confidence intervals centered on the null value (Table S1). For cord blood cadmium, multivariable-adjusted risk ratios were identical in the two highest treatment

Table 4. Intent-to-treat analysis of relative metal levels in maternal (*n* = 619) and neonatal cord (*n* = 516) blood samples, compared with placebo.

Metal	Treatment group						<i>p</i> -Value
	4,200 IU/wk		16,800 IU/wk		28,000 IU/wk		
	<i>n</i>	Percentage difference (95% CI)	<i>n</i>	Percentage difference (95% CI)	<i>n</i>	Percentage difference (95% CI)	
Lead							
Maternal ^a	141	6.3 (−4.1, 18)	121	7.4 (−3.5, 20)	239	6.0 (−3.4, 16)	0.54
Cord	104	8.5 (−3.5, 22)	111	16 (3.3, 30)	201	11 (0.4, 23)	0.08
Mercury							
Maternal	141	−3.7 (−14, 8.0)	121	1.9 (−9.5, 15)	239	−4.2 (−14, 6.2)	0.61
Cord	104	4.3 (−7.0, 17)	111	7.5 (−4.1, 20)	201	6.3 (−3.9, 18)	0.60
Manganese							
Maternal	141	1.0 (−6.5, 9.0)	121	3.9 (−4.0, 13)	239	0.1 (−6.6, 7.2)	0.72
Cord	104	−2.5 (−11, 6.3)	111	1.5 (−6.8, 11)	201	3.9 (−3.8, 12)	0.40
Cadmium							
Maternal	141	6.6 (−5.0, 20)	121	4.9 (−7.0, 18)	239	1.6 (−8.4, 13)	0.67
Cord ^b	104	2.2 (1.3, 3.7)	111	1.4 (0.8, 2.5)	201	1.7 (1.0, 2.9)	0.02

Note: Placebo group: maternal blood samples, *n* = 118, cord blood samples, *n* = 100. CI, confidence interval; LOD, limit of detection.

^aOne sample below the LOD was imputed as the LOD (0.1 μg/L).

^b76% of samples were undetectable, relative risk (95% CI) of detectable cadmium.

Table 5. Spearman correlation coefficients and 95% confidence intervals between log-transformed metals and 25OHD measures.

	Change in 25OHD	Maternal lead	Maternal manganese	Maternal mercury	Maternal cadmium	Cord lead	Cord manganese	Cord mercury
Baseline 25OHD ^a	-0.19 (-0.28, -0.11)	0.0056 (-0.07, 0.08)	-0.01 (-0.09, 0.07)	0.21 (0.13, 0.28)	-0.04 (-0.12, 0.04)	-0.06 (-0.14, 0.02)	-0.03 (-0.11, 0.06)	0.15 (0.06, 0.23)
Change in 25OHD ^b	1	0.07 (-0.03, 0.17)	-0.0007 (-0.10, 0.10)	0.06 (-0.04, 0.17)	-0.06 (-0.17, 0.04)	0.11 (0.02, 0.20)	0.01 (-0.08, 0.11)	0.03 (-0.06, 0.13)

Note: 76% of cord blood cadmium samples were undetectable, so correlations were not calculated. 25OHD, 25-hydroxyvitamin D.

^aFor correlation between baseline 25OHD and change in 25OHD, $n = 482$; for baseline 25OHD and maternal metals, $n = 616$; and for baseline 25OHD and cord blood metals, $n = 513$.

^bFor the correlation between change in 25OHD and maternal metals, $n = 368$; and for change in 25OHD and cord blood metals, $n = 422$.

groups and only slightly smaller in the lowest treatment group [RR = 2.0 (95% confidence interval: 1.1, 3.6)] (Table S1).

Our data did not suggest large differences in vitamin D treatment effects when stratified by BMI. Treatment effects did not differ among women who were normal or underweight ($n = 335$), compared with overweight or obese women ($n = 227$) for either maternal lead levels ($p_{\text{interaction}} = 0.69$) or infant cord blood lead levels ($p_{\text{interaction}} = 0.93$) (Table 6).

Discussion

In this randomized placebo-controlled trial of prenatal vitamin D beginning in the second trimester of pregnancy, whole-blood metal concentrations at the time of delivery did not differ between women who received vitamin D supplementation compared with placebo. For lead, the vitamin D treatment effect estimates were in the hypothesized direction but were small with confidence intervals that included the null. Women who received supplementation had infants who were more likely to have detectable cord blood cadmium levels and to have higher cord blood lead levels, particularly the women in the two highest treatment groups (16,800 and 28,000 IU). Accounting for baseline 25OHD or adherence to the assigned treatment regimen did not change our conclusions.

We hypothesized that vitamin D supplementation would stimulate an increase in maternal toxic metal absorption, leading to higher whole-blood metal concentrations in women who received vitamin D supplements. Treatment with active vitamin D, 1,25 dihydroxyvitamin D, has been shown to increase lead absorption in rats and chicks, particularly if they were deficient (Moon 1994). 1,25 dihydroxyvitamin D, stimulates the expression of both membrane calcium channels [e.g., transient receptor potential channel superfamily, vanilloid family, 6 (TRPV6)] in the intestine and calcium-binding proteins (or calbindins), which work together to absorb calcium (Christakos et al. 2017). TRPV6 is also found in the placenta (Kovacs et al. 2011) and may provide a pathway for calcium to enter the fetus. TRPV6 (Kovacs et al. 2011) and calbindins (Wasserman and Corradino 1973) can also bind to cadmium and to lead, potentially with higher affinity (Fullmer et al. 1985). This suggests that toxic metals may follow active transcellular calcium absorption and fetal transfer pathways. At the same time, this does not preclude the possibility of other absorption pathways. Animal studies indicate increases in metal levels can occur through additional pathways that do not depend on calcium transporters (Moon 1994). For example, calcium can also be absorbed through a passive process that occurs across intercellular spaces when intraluminal concentrations are high (Christakos et al. 2017) and vitamin D can regulate proteins that influence the space between intestinal cells (Christakos et al. 2017). These pathways focus on lead and cadmium, and they may be applicable to mercury and manganese given that they are also divalent cations. The literature investigating mercury and manganese is sparse. One study has reported no effect of vitamin D on mercury absorption (Masuhara and Migicovsky 1963). In a colorectal carcinoma cell line, active vitamin D treatment increased manganese transporter gene and protein expression (Claro da Silva et al. 2016).

Our study was limited by a single measurement of metals, and we were unable to investigate longitudinal changes in maternal metal levels across pregnancy that might have resulted from vitamin D treatment. The half-life of blood lead in adults is ~28–36 d (ATSDR 2019), and treatment in this study spanned an ~20-wk window. Moreover, previous studies of maternal blood lead across pregnancy show either a U-shaped or increasing curve toward the third trimester, when calcium requirements increase and bone is broken down, liberating stored lead (ATSDR 2007). The nadir in blood

Table 6. Analysis of relative lead levels in maternal and neonatal cord blood samples compared with placebo, stratified by BMI.

		Treatment group (IU/wk)				
		4,200	16,800	28,000		
Metal	<i>n</i>	Geometric mean	Percentage difference (95% CI)	Percentage difference (95% CI)	Percentage difference (95% CI)	<i>p</i> -Value ^a
Maternal lead						
Low/normal BMI	335	8.4	8.4 (−5.6, 24)	12 (−3.8, 29)	6.7 (−6.1, 21)	0.70
Overweight/obese	227	9.6	12 (−5.6, 33)	3.7 (−12, 22)	10 (−5.2, 29)	
Cord blood lead						
Low/normal BMI	304	7.3	13 (−3.1, 31)	20 (3.0, 40)	20 (5.0, 37)	0.93
Overweight/obese	179	7.6	8.8 (−12, 34)	17 (−2.8, 42)	12 (−6.4, 34)	

Note: BMI, body mass index; CI, confidence interval.

^aLikelihood ratio p-value testing the interaction between BMI and vitamin D treatment group.

lead is at ~20 wk of gestation (Gulson et al. 2016). Women entered our study around this time, and, thus, if vitamin D has no effect, we would expect blood lead levels to increase equally across gestation in all groups. Calcium supplementation changes the pattern of blood lead increase across gestation (Gulson et al. 2016), and it is not clear how vitamin D and calcium together may influence this pattern. Vitamin D may simultaneously reduce bone lead liberation but stimulate lead uptake, potentially resulting in small net effects on maternal blood lead. A further limitation is that detailed, objective information regarding adherence to the calcium supplementation was not collected prospectively during the trial, although women's self-reported information suggests high adherence.

Additional limitations of our study include that BMI was measured at 12 months postpartum given that no measure of pre-pregnancy BMI was collected. Metals were measured only on a subset of the trial participants because some specimens were either missing or unsuitable for analysis. This can lead to selection bias if those that are measured differ from the full cohort. We found some difference between the groups with respect to BMI and month of enrollment; however, analyses accounting for these factors did not alter our conclusions. Although we found suggestive evidence of differences in metal levels by water source, the accuracy of reports of water source is uncertain, and it is unclear how reported water source in this setting might reflect water quality or metal levels more directly. Finally, the use of blood manganese as a biomarker of manganese exposure has been questioned (ATSDR 2012b), and individual variability in blood manganese levels may have limited our ability to detect an association between vitamin D treatment and manganese level.

For the most part, we did not see increases in maternal metal levels with vitamin D treatment; however, there were increases in neonatal cord blood lead and cadmium in the treatment groups. In a previous study by this research group, maternal vitamin D supplementation (without calcium) was associated with small increases in maternal serum calcium at delivery (0.02 mmol/L) and neonatal cord blood calcium levels (0.05 mmol/L) (Harrington et al. 2014). These changes are small, most likely due to the tight regulation of calcium in adult blood (between 2.2 and 2.7 mmol/L) (Goldstein 1990). However, the direction of the observed associations agrees with the results of one study of sheep that reported an increase in calcium levels in fetal tissues with maternal vitamin D supplementation, moreover plasma levels of calcium were higher in the fetus compared with the maternal plasma, suggesting active transfer of calcium across the placenta (Durand et al. 1983). It is possible that toxic metals are analogously transferred across the placenta through molecular mimicry. As with calcium, this might also lead to higher metals levels in the fetal compartment than in the maternal circulation. In our sample, maternal and neonatal cord blood metal levels were correlated. Given that we did not observe increases in neonatal cord blood mercury and manganese, these placental transfer mechanisms may not be equally accessible across metals. It is

also possible that vitamin D supplementation increases calcium absorption or affects circulating calcium levels, which in turn affect metal levels. One previous cross-sectional study reported that calcium intake was inversely related to maternal lead level during pregnancy (Lee 2013). In our study population, we saw that vitamin D treatment slightly increased serum calcium levels, with little effect on maternal lead levels and a larger increase in cord blood lead levels. Thus, although the increase in calcium we observed was not associated with a reduction in circulating lead, serum calcium may be a mediator on the pathway between vitamin D and metals levels.

Few studies have examined the correlations between vitamin D treatment and metal levels. In a small study of HIV-positive children and adolescents ($n=44$), vitamin D treatment was not associated with increased blood lead levels, and increased 25OHD was associated with a decrease in blood lead levels (Groleau et al. 2013). The generalizability of these findings is unclear, given the potential role of HIV and antiretroviral therapy on bone disease (Gediminas and Solomon 2012). One randomized trial of calcium supplementation during pregnancy reported decreased blood lead levels among Mexican mothers who received 1,200 mg/d of calcium (Ettinger et al. 2009). Observational studies have reported inverse associations between maternal dietary intake of vitamin D and cord blood lead levels (Schell et al. 2000), maternal cadmium and lead (Arbuckle et al. 2016), or cadmium levels (Suh et al. 2016). A National Health and Nutrition Examination Survey study reported no correlation between 25OHD and blood lead in nonpregnant women (Jackson et al. 2010). We did not find any studies investigating associations between vitamin D intake and mercury or manganese. It is possible that higher dietary vitamin D intake is associated with higher calcium intake, which can block the absorption of toxic metals (Goyer 1997; Moon 1994). Observational studies are difficult to interpret, however, given that some have assessed vitamin D through self-reported diet without biomarkers of vitamin D. In addition, the observational design limits causal inference given that it has also been reported that higher exposure to metals influences vitamin D metabolism and function (Moon 1994). Given the interaction between toxic metals and vitamin D, a randomized trial is the ideal study design for detecting changes in circulating levels of metals that result from vitamin D supplementation.

Our study is the first to examine differences in circulating lead, cadmium, manganese, and mercury in pregnant women participating in a randomized trial of vitamin D. Compared with the placebo group, vitamin D–treatment groups had higher cord blood lead concentrations with no evidence of a dose–response relationship. The frequency of detectable cord blood cadmium was also higher in the treatment groups. These associations were not seen in maternal blood. Future research regarding maternal vitamin D supplementation should investigate whether there are transient increases in maternal metal levels across pregnancy that are resolved prior to delivery. In addition, future research should

examine whether the increase in cord blood metals persists or whether this increase is temporary either with supplementation or while *in utero*. Finally, all women in this trial were supplemented with calcium in addition to vitamin D. It is well known that calcium supplementation can prevent toxic metal absorption (CDC 2002; Goyer 1997), which may have reduced any direct effect of vitamin D supplementation on metals levels. It is possible that higher levels of calcium supplementation are required with higher levels of vitamin D supplementation to fully prevent the absorption of toxic metals. The relationship between vitamin D supplementation, calcium supplementation, and metals levels should be further studied. There are no safe levels of metal exposures and any increase caused by vitamin D supplementation requires further exploration.

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